DRAFT: August 31, 1994

DECISION DOCUMENT
TSCA SECTION 5(H)(4) EXEMPTION FOR
SACCHAROMYCES CEREVISIAE

I. SUMMARY

Saccharomyces cerevisiae has an extensive history of use in the area of food processing. Also known as Baker's Yeast or Brewer's Yeast, this organism has been used for centuries as leavening for bread and as a fermenter of alcoholic beverages. With a prolonged history of industrial applications, this yeast has been either the subject of or model for various studies in the principles of microbiology. Jacob Henle based his theories of disease transmission on studies of strains of Brewer's Yeast. Currently, S. cerevisiae is the subject of a major international effort to characterize a eucaryotic genome.

II. BACKGROUND

A. Introduction

EPA recognizes that some microorganisms present a low risk when used under specific conditions at general commercial use. Therefore, EPA is proposing expedited regulatory processes for certain microorganisms under these specific conditions at the general commercial use stage. Microorganism uses that would be exempt meet criteria addressing: (1) performance based standards for minimizing the numbers of microorganisms emitted from the manufacturing facility; (2) the introduced genetic material; and (3) the recipient microorganism. Microorganisms that qualify for these exemptions, termed Tier I and Tier II, must meet a standard of no unreasonable risk in the exempted use.

To evaluate the potential for unreasonable risk to human health or the environment in developing these exemptions, EPA focuses primarily on the characteristics of the recipient microorganisms. If the recipient is shown to have little or no potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase potential for adverse effects. As further assurance that risks would be low, EPA is also specifying procedures for minimizing numbers of organisms emitted from the facility. When balanced against resource savings for society and expected product benefits, these exemptions will not present unreasonable risks.

B. Criteria for Minimizing Release from Manufacturing Facilities

The standards prescribed for the Tier I exemption require the following: (1) the structure(s) be designed and operated to contain the microorganism, (2) access to the structure should be limited to essential personnel, (3) inactivation procedures shown to be effective in reducing the number of viable microorganisms in liquid and solid wastes should be followed prior to disposal of the wastes, (4) features to reduce microbial concentrations in aerosols and exhaust gases released from the structure should be in place, and (5) general worker hygiene and protection practices should be followed.

1. Definition of structure. EPA considers the term "structure" to refer to the building or vessel which effectively surrounds and encloses the microorganism. Vessels may have a variety of forms, e.g., cubic, ovoid, cylindrical, or spherical, and may be the fermentation vessel proper or part of the downstream product separation and purification line. All would perform the function of enclosing the microorganism. In general, the material used in the construction of such structure(s) would be impermeable, resistant to corrosion and easy to clean/sterilize. Seams, joints, fittings, associated process piping, fasteners and other similar elements would be sealed.

2. Standards to minimize microbial release. EPA is proposing, for several reasons, a somewhat cautious approach in prescribing standards for minimizing the number of microorganisms emitted through the disposal of waste and the venting of gases. First, a wide range of behaviors can be displayed by microorganisms modified consistent with EPA's standards for the introduced genetic material. Second, EPA will not conduct any review whatsoever for Tier I exemptions. EPA believes the requirement to minimize emissions will provide a measure of risk reduction necessary for making a finding of no unreasonable risk. Taken together, EPA's standards ensure that the number of microorganisms emitted from the structure is minimized.

EPA's proposed standards for minimizing emission specify that liquid and solid waste containing the microorganisms be treated to give a validated decrease in viable microbial populations so that at least 99.9999 percent of the organisms resulting from the fermentation will be killed. Since the bacteria used in fermentation processes are usually debilitated, either intentionally or through acclimation to industrial fermentation, the small fraction of microorganisms remaining viable after inactivation treatments will likely have a reduced ability to survive during disposal or in the environment.

Moreover, industrial companies, in an attempt to keep their proprietary microorganisms from competitors and to reduce the microbial numbers to those permitted by local sanitation authorities, modify the microorganisms to increase the ability of their microorganisms to survive and perform their assigned tasks in the fermentor but decrease their ability to survive in the environment external to the fermentor.

EPA requirements also address microorganisms in the exhaust from the fermentor and along the production line. To address exhaust from fermentors, EPA is proposing that the number of microorganisms in fermentor gases be reduced by at least two logs prior to the gases being exhausted from the fermentor. EPA selected this number based on an estimate of the numbers of microorganisms likely to be in the exhaust from an uncontrolled fermentor and common industry practice. Moreover, microorganisms that are physiologically acclimated to the growth conditions within the fermentor are likely to be compromised in their ability to survive aerosolization. EPA anticipates, therefore, that few microorganisms will survive the stresses of aerosolization associated with being exhausted in a gas from the fermentor. The provision requiring reduction of microorganisms in fermentor exhaust gases contributes to minimizing the number of viable microorganisms emitted from the facility.

EPA is also proposing that the requirements specify that other systems be in place to control dissemination of microorganisms by other routes. This would include programs to control pests such as insects or rats, since these might serve as vectors for carrying microorganisms out of the fermentation facilities.

3. Worker protection. The requirement to minimize microbial emissions, in conjunction with the requirement for general worker safety and hygiene procedures, also affords a measure of protection for workers. Potential effects on workers that exist with microorganisms in general (e.g., allergenicity) will be present with the microorganisms qualifying for this exemption. As with other substances that humans may react to (e.g., pollen, chemicals, dust), the type and degree of allergenic response is determined by the biology of the exposed individual. It is unlikely that a microorganism modified in keeping with EPA's specifications for the introduced genetic material would induce a heightened response. The general worker hygiene procedures specified by EPA should protect most individuals from the allergenic responses associated with microorganisms exhausted from fermentors and/or other substances emitted along the production line. The EPA requirement that entry be limited to essential personnel also addresses this

consideration by reducing to a minimum the number of individuals exposed.

4. Effect of containment criteria. As further assurance that risks would be low, EPA is specifying procedures for m;inimizing numbers of organisms emitted from the facility for the Tier I exemption. EPA is not specifying standards for minimizing the number of microorganisms emitted from the facility for microorganisms qualifying for Tier II exemption. Rather, the Agency requests that submitters utilize as guidance the standards set forth for Tier I procedures. The procedures proposed by the submitter in a Tier II exemption request will be reviewed by the Agency. EPA will have the opportunity to evaluate whether the procedures the submitter intends to implement for reducing the number of organisms emitted from the facility are appropriate for that microorganism.

C. Introduced Genetic Material Criteria

In order to qualify for either Tier I or Tier II exemption, any introduced genetic material must be limited in size, well characterized, free of certain nucleotide sequences, and poorly mobilizable.

1. Limited in size. Introduced genetic material must be limited in size to consist only of the following: (1) the structural gene(s) of interest; (2) the regulatory sequences permitting the expression of solely the gene(s) of interest; (3) the associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites; (4) the nucleotide sequences needed for vector transfer; and (5) the nucleotide sequences needed for vector maintenance.

The limited in size criterion reduces risk by excluding the introduction into a recipient of extraneous and potentially uncharacterized genetic material. The requirement that the regulatory sequences permit the expression solely of the structural gene(s) of interest reduces risk by preventing expression of genes downstream of the inserted genetic material. The limitation on the vector sequences that are components of the introduced genetic material prevents the introduction of novel traits beyond those associated with the gene(s) of interest. The overall result of the limited in size criterion is improved ability to predict the behavior of the resulting microorganism.

2. Well characterized. For introduced genetic material, well characterized means that the following have been determined: (1) the function of all of the products expressed from the

structural gene(s); (2) the function of sequences that participate in the regulation of expression of the structural gene(s); and (3) the presence or absence of associated nucleotide sequences.

Well characterized includes knowledge of the function of the introduced sequences and the phenotypic expression associated with the introduced genetic material. Genetic material which has been examined at the restriction map or sequence level, but for which a function or phenotypic trait has not yet been ascribed, is not considered well characterized. Well characterized would include knowing whether multiple reading frames exist within the operon. This relates to whether more than one biological product might be encoded by a single sequence, and addresses the possibility that a modified microorganism could display unpredicted behavior should such multiple reading frames exist and their action not be anticipated.

3. Free of certain sequences. In addition to improving the ability to predict the behavior of the modified microorganism, the well characterized requirement ensures that segments encoding for either part or the whole of the toxins listed in the proposed regulatory text for the TSCA biotechnology rule would not inadvertently be introduced into the recipient microorganism.

These toxins are polypeptides of relatively high potency. Other types of toxins (e.g., modified amino acids, heterocyclic compounds, complex polysaccharides, glycoproteins, and peptides) are not listed for two reasons. First, their toxicity falls within the range of moderate to low. Second, these types of toxins generally arise from the activity of a number of genes in several metabolic pathways (multigenic).

In order for a microorganism to produce toxins of multigenic origin, a large number of different sequences would have to be introduced and appropriately expressed. It is unlikely that all of the genetic material necessary for metabolizing multigenic toxins would be inadvertently introduced into a recipient microorganism when requirements that the genetic material be limited in size and well characterized are followed.

Similarly, other properties that might present risk concerns result from the interactive expression of a large number of genes. For example, pathogenic behavior is the result of a large number of genes being appropriately expressed. Because of the complex nature of behaviors such as pathogenicity, the probability is low that an insert consisting of well characterized, limited in size genetic material could transform

the microorganisms proposed for exemption into microorganisms which display pathogenic behavior.

4. Poorly mobilizable. Poorly mobilizable means the ability of the introduced genetic material to be transferred and mobilized is inactivated, with a resulting frequency of transfer of less than 10^{-8} transfer events per recipient. The requirement that the introduced genetic material be poorly mobilizable reduces potential for transfer of introduced genetic sequences to other microorganisms in the environment. Such transfers would occur through the interaction of the introduced microorganism with indigenous microorganisms through conjugation, transduction, or transformation. Through such transfers, the introduced genetic material could be transferred to and propagated within different populations of microorganisms, including microorganisms which may never previously have been exposed to this genetic material. It is not possible to predict how the behavior of these potential recipient microorganisms will be affected after uptake and expression of the genetic material.

Since EPA is not limiting the type of organism that can serve as the source for the introduced genetic material, some limitation is placed on the ability of the introduced genetic material to be transferred. This limitation mitigates risk by significantly reducing the probability that the introduced genetic material would be transferred to and expressed by other microorganisms.

The 10^{-8} frequency is attainable given current techniques. Plasmids with transfer rates of 10^{-8} exist or are easily constructed. Some of the plasmids most commonly employed as vectors in genetic engineering (e.g., pBR325, pBR322) have mobilization/transfer frequencies of 10^{-8} or less.

The criteria set for "poorly mobilizable" for transduction and transformation should not prevent most microorganisms from meeting the exemption criteria, since the majority of transfer frequencies reported for transduction and natural transformation are less than 10^{-8}. Higher frequencies are likely only if the introduced genetic material has been altered or selected to enhance frequency.

Fungal gene transfer has also been considered in development of the poorly mobilizable criterion. Although mobile genetic elements such as transposons, plasmids and double stranded RNA exist in fungi and can be readily transferred, this transfer usually is only possible between members of the same species during anastomosis, a process specific to fungi. Since anastomosis only occurs between members of the same species, the

introduced genetic material would not be transferred to distantly related fungi as may occur with bacteria.

5. Effect of introduced genetic material criteria. The requirements placed on the introduced genetic material, in concert with the level of safety associated with Saccharoymyces ceremiviae, ensure that the resulting microorganisms present low or negligible risk. The probability is low that the insertion of genetic material meeting EPA's criteria into strains of S. cerevisiae will change their behavior so that they would acquire the potential for causing adverse effects. Risks would be mitigated by the four criteria placed on the introduced genetic material, the relative safety of S. cerevisiae, and the inactivation criteria specified for the Tier I exemption. In the case of Tier II exemption, risks would be mitigated in light of the four criteria placed on introduced genetic material, the relative safety of the S. cerevisiae, and EPA's review of the conditions selected.

D. Recipient Microorganism Criteria

Six criteria were used by EPA to determine eligibility of recipient microorganisms for the tiered exemption. Microorganisms which EPA finds meet these criteria are listed as eligible recipients. The first criteria would require that it be possible to clearly identify and classify the microorganism. Available genotypic and phenotypic information should allow the microorganism to be assigned without confusion to an existing taxon which is easily recognized. Second, information should be available to evaluate the relationship of the microorganism to any other closely related microorganisms which have a potential for adverse effects on human health or the environment. Third, there should be a history of commercial use for the microorganism. Fourth, the commercial uses should indicate that the microorganism products might be subject to TSCA jurisdiction. Fifth, studies are available which indicate the potential for the microorganism to cause adverse effects on human health and the environment. Sixth, studies are available which indicate the survival characteristics of the microorganism in the environment.

After each microorganism was reviewed using the six evaluation criteria, a decision was made as to whether to place the microorganism on the list. The Agency's specific determination for Saccharomyces cerevisiae is discussed in the next unit.

III. EVALUATION OF SACCHAROMYCES CEREVISIAE

A. History of Use

1. History of safe commercial use. Saccharomyces cerevisiae has an extensive history of use in the area of food processing. Also known as Baker's Yeast or Brewer's Yeast, this organism has been used for centuries as leavening for bread and as a fermenter of alcoholic beverages. In addition to its use in food processing, the organism is widely used for the production of macromolecular cellular components such as lipids, proteins including enzymes, and vitamins. The Food and Drug Administration rates Brewer's Yeast extract as Generally Recognized as Safe. Furthermore, the National Institutes of Health in its Guidelines for Research Involving Recombinant DNA Molecules considers S. cerevisiae a safe organism. Most experiments involving S. cerevisiae have been exempted from the NIH Guidelines based on an analysis of safety (see Appendix C-II of the NIH Guidelines). While alcoholic beverages, vitamins, and bread leavening are covered under the Federal Food, Drug and Cosmetic Act, the production of enzymes and other macromolecules may be subject to TSCA regulation. The abundance of information on S. cerevisiae, derived from its role in industry, has positioned it as a primary model for genetic studies and, by extension, as a strong candidate for genetic manipulation for TSCA applications.

2. Products subject to TSCA jurisdiction. While alcoholic beverages, vitamins, and bread leavening are covered under the Federal Food, Drug and Cosmetic Act, the production of enzymes and other macromolecules may be subject to TSCA regulation. The abundance of information on S. cerevisiae, derived from its role in industry, has positioned it as a primary model for genetic studies and, by extension, as a strong candidate for genetic manipulation for TSCA applications.

B. Identification of the Microorganism

1. Classification. Saccharomyces cerevisiae is a yeast. The organism can exist either as a single-celled organism or as pseudomycelia. The cells reproduce by multilateral budding. It produces from one to four ellipsoidal, smooth-walled ascospores. S. cerevisiae can be differentiated from other yeasts based on growth characteristics and physiological traits: principally the ability to ferment individual sugars. Clinical identification of yeast is conducted using commercially available diagnostic kits which classify the organism through analysis of the ability of the yeast to utilize distinct carbohydrates as sole sources of carbon. More recently, developments in systematics have led to the design of sophisticated techniques for classification, including gas-liquid chromatography of lysed whole cells.

With the use of more sophisticated techniques, what had been classified as one large heterogeneous species, S. cerevisiae, may be broken out into four distinct species based on DNA homology studies. The four species are S. cerevisiae, S. bayanus (also known as S. uvarum), S. pasteurianus (also known as S. carlsbergensis), and S. paradoxus. All four represent industrially important species.

2. Related species of concern. None of the four species created from the original herterogeneous species designated as S. cerevisiae or other closely related species has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment.

C. Risk Summary

1. Studies regarding potential for adverse effects. S. cerevisiae is a organism which has an extensive history of safe use. Despite considerable use of the organism in research and the presence of S. cerevisiae in food, there are few reports in the literature of pathogenicity to humans or animals, and only in those cases where the human had a debilitating condition. Tests for the factors associated with the virulence of yeasts (i.e., phospholipases) indicate that this organism is nonpathogenic. The organism has not been shown to produce toxins to humans.

2. Studies regarding survival in the environment. S. cerevisiae is ubiquitous in nature. It has been recovered from a variety of sites under varying ecological conditions. The organism is used in a variety of industrial scenarios. S. cerevisiae is commonly recovered from a variety of fresh fruits and vegetables, generally those fruits with high levels of fermentable sugars. However, it is not listed as the causative agent of food spoilage for fruits and vegetables. The only adverse effect to the environment noted in the literature is the presence of the "killer toxins" which are active against other strains of Saccharomyces.

IV. BENEFITS SUMMARY

Substantial benefits are associated with this proposed exemption. Saccharomyces cerevisiae is already widely employed in general commercial uses, some of which are subject to TSCA reporting. The Agency believes this exemption will result in resource savings both to EPA and industry without compromising the level of risk management afforded by the full 90 day review. In addition to assessing the risk of S. cerevisiae, EPA has developed criteria limiting the potential for transfer of and

expression of toxin sequences, and the conditions of use specified in the exemption are met (Tier I) or will be reviewed by EPA to ensure adequate risk reduction (Tier II). EPA requirements for minimizing numbers of viable microorganisms emitted are within standard operating procedures for the industry, and both the procedures and the structures specified in the exemption are the type industry uses to protect their products from contamination.

The exemption will result in reduced reporting costs and a decrease in delay associated with reporting requirements. The savings in Agency resources can be directed to reviewing activities and microorganisms which present greater uncertainty. This exemption should also facilitate development and manufacturing of new products and the accumulation of useful information.

V. RECOMMENDATION AND RATIONALE

A. Recommendation

Saccharomyces cerevisiae is recommended for a TSCA section 5(h)(4) tiered exemption.

B. Rationale

1. Risks from use of the recipient microorganism S. cerevisiae are low. There is an extensive history of use of and exposure to S. cerevisiae with a very limited record of adverse effects to the environment or human health. The current taxonomy of Saccharomyces is under revision based on the development of alternative criteria. Data suggests that only with the ingestion of high levels of S. cerevisiae or with the use of immunosuppressives can S. cerevisiae colonize in the body. Even under those conditions, there were no noted adverse effects. Releases of this microorganism to the environment through fermentation uses would not pose any significant ecological hazards, because this microorganism is ubiquitous in the environment and it is not pathogenic to animals or plants.

2. Use of strains of S. cerevisiae which are eligible for the TSCA section 5(h)(4) exemption present no unreasonable risk. The current taxonomy of Saccharomyces is under revision based on the development of alternative criteria. However, this should not have a major effect on the risk associated with closely related species. Saccharomyces, as a genus, present low risk to human health or the environment. Criteria used to differentiate

between species are based on their ability to utilize specific carbohydrates without relevance to pathogenicity. Nonetheless, this exemption applies to those organisms that fall under the classical definition of S. cerevisiae as described by van der Walt. As part of their eligibility for this TSCA section 5(h)(4) exemption, companies are required to certify that they are using S. cerevisiae. It is therefore expected that companies will have information in their files which documents the correct identification of their strains.

Because the recipient microorganism was found to have little potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase potential for adverse effects. As further assurance that risks would be low, EPA is specifying procedures for minimizing numbers of organisms emitted from the facility for the Tier I exemption and will be reviewing the conditions selected for the Tier II exemption. When balanced against resource savings for society and expected product benefits, this exemption will not present unreasonable risks.

Attachment 1:

INTEGRATED RISK ASSESSMENT OF SACCHAROMYCES CEREVISIAE

I. INTRODUCTION

Saccharomyces cerevisiae has an extensive history of use in the area of food processing. Also known as Baker's Yeast or Brewer's Yeast, this organism has been used for centuries as leavening for bread and as a fermenter of alcoholic beverages. With a prolonged history of industrial applications, this yeast has been either the subject of or model for various studies in the principles of microbiology. Jacob Henle based his theories of disease transmission on studies of strains of Brewer's Yeast. Currently, S. cerevisiae is the subject of a major international effort to characterize a eucaryotic genome (Anderson, 1992).

History of Commercial Use and Products Subject to TSCA Jurisdiction

Saccharomyces cerevisiae, in addition to its use in food processing, is widely used for the production of macromolecular

cellular components such as lipids, proteins including enzymes, and vitamins (Bigelis, 1985; Stewart and Russell, 1985).

The Food and Drug Administration rates Brewer's Yeast extract as Generally Recognized as Safe (FDA, 1986). Furthermore, the National Institutes of Health in its Guidelines for Research Involving Recombinant DNA Molecules (DHHS, 1986) considers S. cerevisiae a safe organism. Most experiments involving S. cerevisiae have been exempted from the NIH Guidelines based on an analysis of safety (see Appendix C-II of the NIH Guidelines). While alcoholic beverages, vitamins, and bread leavening are covered under the Federal Food, Drug and Cosmetic Act, the production of enzymes and other macromolecules may be subject to TSCA regulation. The abundance of information on S. cerevisiae, derived from its role in industry, has positioned it as a primary model for genetic studies and, by extension, as a strong candidate for genetic manipulation for TSCA applications (Dynamac, 1990).

II. IDENTIFICATION AND CLASSIFICATION

A. Taxonomy and Characterization

Saccharomyces cerevisiae is a yeast. The organism can exist either as a single-celled organism or as pseudomycelia. The cells reproduce by multilateral budding. It produces from one to four ellipsoidal, smooth-walled ascospores. S. cerevisiae can be differentiated from other yeasts based on growth characteristics and physiological traits: principally the ability to ferment individual sugars. Clinical identification of yeast is conducted using commercially available diagnostic kits which classify the organism through analysis of the ability of the yeast to utilize distinct carbohydrates as sole sources of carbon (Buesching et al., 1979; Rosini et al., 1982). More recently, developments in systematics have led to the design of sophisticated techniques for classification, including gas-liquid chromatography of lysed whole cells (Brondz and Olsen, 1979).

As a result of the application of newer techniques arising from innovative approaches, the taxonomy of Saccharomyces is subject to greater scrutiny. The initial classification was based principally on morphological characteristics with specific physiological and biochemical traits used to differentiate between isolates with similar morphological traits. Using these criteria, there are as many as 18 species listed in the literature. In addition, what had been classified as one large heterogeneous species, S. cerevisiae, may, in the future, be divided into four distinct species based on DNA homology studies. The four species are S. cerevisiae, S. bayanus (also known as S.

uvarum), S. pasteurianus (also known as S. carlsbergensis), and S. paradoxus. All four represent industrially important species. None of these organisms or other closely related species has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment.

Any assessment of Saccharomyces must take into consideration the malleability of the current classification. For this assessment of S. cerevisiae the reviews of the organism are based on the classification proposed by Van der Walt (1971).

B. Related Species of Concern

None of the above strains or other closely related species has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment.

III. HAZARD ASSESSMENT

A. Human Health Hazards

1. Colonization and Pathogenicity

S. cerevisiae is a commonly used industrial microorganism and is ubiquitous in nature, being present on fruits and vegetables. Industrial workers and the general public come into contact with S. cerevisiae on a daily basis through both inhalation and ingestion (see section IV). Saccharomyces spp. are frequently recovered from the stools and throats of normally healthy individuals. This indicates that humans are in constant contact with these yeasts.

There are individuals who may ingest large quantities of S. cerevisiae every day, for example, people who take the yeast as part of a "health food" regimen. Therefore, studies were conducted to ascertain whether the ingestion of large numbers of these yeasts might result in either colonization, or colonization and secondary spread to other organs of the body. It was found that the installation of very large numbers of S. cerevisiae into the colons of animals would result in both colonization and passage of the yeasts to draining lymph nodes. It required up to 10^{10} S. cerevisiae in a single oral treatment to rats to achieve a detectable passage from the intestine to the lymph nodes (Wolochow et al., 1961). The concentrations of S. cerevisiae required were well beyond those that would be encountered through normal human daily exposure.

S. cerevisiae is not considered a pathogenic microorganism, but has been reported rarely as a cause of opportunistic infections. Eng et al. (1984) described five cases of such infections and reviewed the literature on eight other S. cerevisiae infections (also briefly reviewed by Walsh and Pizzo, 1988). All of the patients in the cases had underlying disease. Some of them had also received antibiotic therapy, thereby suppressing normal bacterial flora and allowing mycotic organisms to become established.

A low concern for the pathogenicity of S. cerevisiae is also illustrated by a series of surveys conducted at hospitals over the last several years. S. cerevisiae accounted for less than 1% of all yeast infections isolated at a cancer hospital and in most of the cases the organism was isolated from the respiratory system (Kiehn et al., 1980). At Yale-New Haven Hospital over the past five years, there have been 50 isolates of S. cerevisiae recovered from patients; however, most of the isolates were considered contaminants (Dynamac, 1991).

2. Toxin Production

There have been no reports of isolates of S. cerevisiae that produce toxins against either humans or animals. However, S. cerevisiae has been shown to produce toxins against other yeasts. These toxins, termed "killer toxins", are proteins or glycoproteins produced by a range of yeasts. The yeasts have been genetically modified to alter activity and are used in industrial settings as a means of controlling contamination of fermentation systems by other yeasts (Sid et al., 1988).

3. Measure of the Degree of Virulence

A number of individual virulence factors have been identified as being associated with the ability of yeasts to cause disease. The principal virulence factors associated with yeasts appear to be phospholipase A and lysophospholipase. It is believed that these enzymes enhance the ability of the yeast to adhere to the cell-wall surface and result in colonization as a first step in the infectious process. Nonpathogenic yeast had considerably lower phospholipase activities. Of a wide range of fungi assayed for phospholipase production, S. cerevisiae was found to have the lowest level of activity (Barrett-Bee et al., 1985). Therefore, based on the phospholipase virulence factor S. cerevisiae is considered a nonpathogenic yeast.

A second factor associated with virulence in yeast is the ability of a fungus to impair the host's immune capabilities. The cell walls of most fungi have the capacity to impede the

immune response of the host. In a study to determine the overall pathogenicity of a number of yeasts used in industrial processes, animals exposed to both high levels of S. cerevisiae and cortisone demonstrated a greater ability of the fungus to colonize compared with those animals treated with only the yeast. However, the animals suffered no ill-effects from exposure to S. cerevisiae, (Holzschu et al., 1979). Therefore, this study suggests that even with the addition of high levels of an immuosuppresant agent, S. cerevisiae appears to be nonpathogenic.

4. Ability to Transfer Virulence Factor Genes

S. cerevisiae does not carry virulence factors to humans or animals. However, the species does carry linear, double-stranded plasmids which can be transmitted to other Saccharomyces. These plasmids carry genes that encode the "killer toxins" discussed above can be transferred from one Saccharomyces to another. Therefore, gene constructs involving the incorporation of traits using these linear plasmids should be considered to be nonstable.

5. Summary

In conclusion, S. cerevisiae is a organism which has an extensive history of safe use. Despite considerable use of the organism in research and the presence of S. cerevisiae in food, there are limited reports in the literature of its pathogenicity to humans or animals, and only in those cases where the human had a debilitating condition. Factors associated with the virulence of yeasts (i.e., phospholipases) indicate that this organism is nonpathogenic. The organism has not been shown to produce toxins to humans.

B. Environmental Hazards

S. cerevisiae is ubiquitous in nature. It has been recovered from a variety of sites under varying ecological conditions. The organism is used in a variety of industrial scenarios. S. cerevisiae is commonly recovered from a variety of fresh fruits and vegetables, generally those fruits with high levels of fermentable sugars. However, it is not listed as the causative agent of food spoilage for fruits and vegetables (Phaff, et al.,1966). The only adverse effect to the environment noted in the literature is the presence of the "killer toxins" which is active against other strains of Saccharomyces.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

S. cerevisiae is considered a Class 1 Containment Agent under the National Institute of Health (NIH) Guidelines for Recombinant DNA Molecules (U.S. Department of Health and Human Services, 1986).

No data were available for assessing the release and survival specifically for fermentation facilities using S. cerevisiae. Therefore, the potential worker exposures and routine releases to the environment from large-scale, conventional fermentation processes were estimated on information available from eight premanufacture notices submitted to EPA under TSCA Section 5 and from published information collected from non-engineered microorganisms (Reilly, 1991). These values are based on reasonable worst-case scenarios and typical ranges or values are given for comparison.

During fermentation processes, worker exposure is possible during laboratory pipetting, inoculation, sampling, harvesting, extraction, processing and decontamination procedures. A typical site employs less than 10 workers/shift and operates 24 hours/day throughout the year. NIOSH has conducted walk-through surveys of several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These particular facilities were not using recombinant microorganisms, but the processes were considered typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest, ie. near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in air sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m^3. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data are not available at the present time. Thus, area sampling data have been the only means of assessing exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day. Assuming that the concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area sampling, the worst-case daily inhalation exposure is estimated to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known (Reilly, 1991).

B. Environmental and General Exposure

1. Fate of the Organism

S. cerevisiae is a normal inhabitant of soils and is widespread in nature. S. cerevisiae is able to take up a wide variety of sugars and amnio acids. These traits enhance the organism's ability for long term survival. S. cerevisiae can be isolated f rom fruits and grains and other materials with a high concentration of carbohydrates (LaVeck, 1991).

2. Releases

Estimates of the number of S. cerevisiae organisms released per production batch are tabulated in Table 1. The minimally controlled scenario assumes no treatment of the fermentor off-gas and assumes 100-fold (2 log) reduction of the maximum cell density of the fermentation broth resulting from inactivation (Reilly, 1991). The containment criteria required for the full exemption scenario assume the use of in-line filters to treat vent gases and a 99% removal efficiency under normal operating conditions. They also assume an overall 6-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation steps (Reilly, 1991).

TABLE 1. Estimated Number of Viable Saccharomyces cerevisiae Organisms Per Production Batch

Release Media	Minimally Controlled (cfu/day)	Full Exemption (cfu/day)	Release (days/year)
Air Vents	$2x10^{8}$ - $1x10^{11}$	$2x10^{6}$ - $1x10^{9}$	350
Rotary Drum Filter	250	250	350
Surface Water	$7x10^{12}$	$7x10^{8}$	90
Soil/Landfill	$7x10^{14}$	$7x10^{10}$	90

Source: Reilly, 1991

3. Air

While there is no specific information on the survival of S. cerevisiae in the atmosphere, the organism's ability to form ascospores suggests that survival rates would be very good. Environmental exposure would occur as the organisms drift to earth and take up residence in the soil. Human exposure is expected to be low, since the numbers of organisms released would be quickly diluted in the atmosphere (LaVeck, 1991).

4. Water

S. cerevisiae released to water would be expected to survive publicly owned treatment works (POTW) treatment and discharge. Surface water concentrations of organisms were estimated using the 10% and 50% flow values for SIC Code 283 (drugs, medicinal chemicals, and pharmaceuticals) that release to surface water. The SIC code flow was estimated using 128 indirect (facilities that send their waste to a POTW) and direct (facilities that have an NPDES permit to discharge to surface water) dischargers. Discharger data were extracted from the IFD (Industrial Facilities Dischargers) databased and surface water flow data were taken from the RXGAGE database, maintained by the EPA. These data, which were partitioned into percentile rankings and flows for the 10th percentile (small river) and 50th (average river), were extracted and used for the exposure calculations. Flow is expressed in Millions of Liters/Day (MLD). Mean Flow is the average flow value, and 7Q10 flow is the lowest flow observed over 7 consecutive days during a 10 year period. Concentrations of microorganisms in surface water are calculated for both the minimally controlled and the full exemption scenarios (LaVeck, 1991).

TABLE 2. Saccharomyces cerevisiae Concentrations in Surface Water

Flow	Receiving Stream Flow (MLD*)		Organisms (cfu/l)	
	Mean	Q710	Mean	Q710
Minimally Controlled				
10th Percentile	159	4.57	4.4×10^{4}	1.53×10^{6}
50th Percentile	768	68.13	9.11×10^{3}	1.03×10^{5}
Full Exemption				
10th Percentile	159	4.57	4.4×10^{0}	1.53×10^{2}
50th Percentile	768	68.13	9.11×10^{-1}	1.03×10^{1}

*MLD = million liters per day
Source: LaVeck, 1991

5. Soil

S. cerevisiae would be expected to survive well in soil. These releases could result in human and environmental exposure. However, there is no evidence to suggest that any problems would

ensue from this exposure. It is currently estimated that over one million tons of yeast are produced annually during brewing and distilling practices (LaVeck, 1991).

V. INTEGRATED RISK ASSESSMENT

A. Discussion

There is an extensive history of use of and exposure to S. cerevisiae with a very limited record of adverse effects to the environment or human health. Yeast has been used for centuries as a leavening for bread and fermenter of beer without records of virulence. S. cerevisiae is currently classified as a class 1 containment organism under the NIH Guidelines based largely on the extensive history of safe use.

Factors associated with the development of disease states in fungi have been reviewed. Data suggests that only with the ingestion of high levels of S. cerevisiae or with the use of immunosuppressives can S. cerevisiae colonize in the body. Even under those conditions, there were no noted adverse effects. In the few cases which S. cerevisiae was found in association with a disease state, the host was a debilitated individual, generally with an impaired immune system. In other cases the organism was recovered from an immunologically privileged site (i.e., respiratory tract). Many scientists believe that under appropriate conditions any microorganism could serve as an opportunistic pathogen. The cases noted in the above Human Health Assessment, where S. cerevisiae was found in association with a disease state, appear to be classic examples of opportunistic pathogenicity (see III.A.3).

The organism is not a plant or animal pathogen. Despite the fact that S. cerevisiae is ubiquitous in nature, it has not been found to be associated with disease conditions in plants or animals. The only adverse environmental condition that was noted is the production of "killer toxins" by some strains of the yeast. These toxins have a target range that is limited to susceptible yeasts. The toxins, proteins and glycoproteins, are not expected to have a broad environmental effect based largely on the anticipated short persistence of the toxins in soil or water and by the limited target range. S. cerevisiae "killer toxin" has been used industrially to provide a level of protection against contamination by other yeasts in the fermentation beer.

The current taxonomy of Saccharomyces is under revision based on the development of alternative criteria. However, this

should not have a major effect on the risk associated with closely related species. Saccharomyces, as a genus, present low risk to human health or the environment. Criteria used to differentiate between species are based on their ability to utilize specific carbohydrates without relevance to pathogenicity. Nonetheless, this risk assessment applies to those organisms that fall under the classical definition of S. cerevisiae as described by van der Walt (1971).

S. cerevisiae is a ubiquitous organism which, despite its broad exposure, has very limited reported incidence of adverse effects. The extensive history of use, the diversity of products currently produced by the organism, and the attention given this organism as a model for genetic studies collectively makes this organism a prime candidate for full exemption. The increased knowledge derived from the ongoing research should further enhance this organisms' biotechnological uses.

B. Recommendation

It is the recommendation of this assessor that Saccharomyces cerevisiae be granted full exemption from regulatory oversight. Because this genus is currently under flux this recommendation holds for those organisms classified as S. cerevisiae according to the characteristics set forth by J. van der Walt (1971).

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